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Taking control of microplastics data: A comparison of control and blank data correction methods

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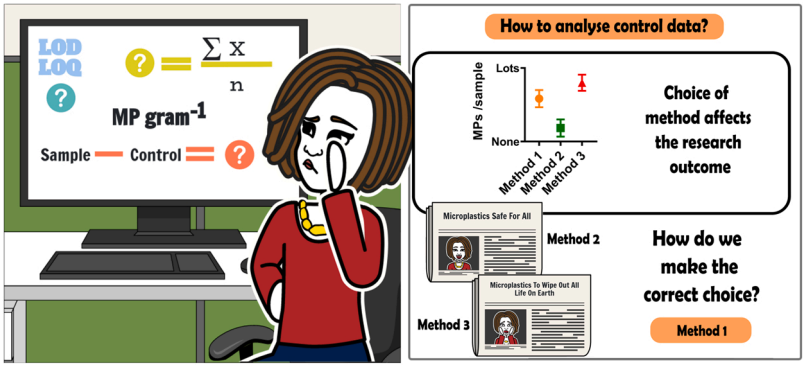
HIGHLIGHTS

- Microplastic research has not reached a consensus on how to analyse control data.
- We tested 51 correction methods to find if currently used methods are valid.
- Only 7 of the 51 methods were found suitable for analysis of microplastic data.
- LOD/LOQ methods are the most reliable for microplastics data.

GRAPHICAL ABSTRACT



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ABSTRACT

Although significant headway has been achieved regarding method harmonisation for the analysis of microplastics, analysis and interpretation of control data has largely been overlooked. There is currently no consensus on the best method to utilise data generated from controls, and consequently many methods are arbitrarily employed. This study identified 6 commonly implemented strategies: a) No correction; b) Subtraction; c) Mean Subtraction; d) Spectral Similarity; e) Limits of detection/ limits of quantification (LOD/LOQ) or f) Statistical analysis, of which many variations are possible. Here, the 6 core methods and 45 variant methods (n = 51) thereof were used to correct a dummy dataset using control data. Most of the methods tested were too inflexible to account for the inherent variation present in microplastic data. Only 7 of the 51 methods tested (six LOD/LOQ methods and one statistical method) showed promise, removing between 96.3 % and 100 % of the contamination data from the dummy set. The remaining 44 methods resulted in deficient corrections for background contamination due to the heterogeneity of microplastics. These methods should be avoided in the future to avoid skewed results, especially in low abundance samples. Overall, LOD/LOQ methods or statistical analysis comparing means are recommended for future use in microplastic studies.

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1. Introduction

After more than a decade of research, the harmonisation of methods has become an international priority for environmental monitoring of microplastics (GESAMP, 2019). This harmonisation has focused predominantly on extraction and isolation methods (Lusher et al., 2020), identification of microplastics (Brandt et al., 2021; Primpke et al., 2020b) and, more recently reporting requirements (Cowger et al., 2020) and quality assurance and quality control (QA/QC). QA/QC is an important step when analysing environmental pollutants, as it determines the accuracy of the measurements taken, facilitating robust repeatable data and distilling trust in the generated results (DES, 2018; Michener, 2018). In addition to removing or reducing background contamination, by, for example, using clean equipment, certified or cleaned reagents, fit-for-purpose laboratories, and adequate procedural training, controls and blanks play an important role in QA/QC measures (ANZG, 2018). However, throughout this period of microplastics method harmonisation, less emphasis has been placed on the appropriate use of controls (Dawson et al., 2021; Hermsen et al., 2018) and how microplastics control data is reported and utilised to correct sample data. The use of controls and blanks is essential when collecting environmental data to characterise background levels of contaminants which may be introduced to the samples throughout the analytical process (e.g. Cowger et al., 2020; Koelmans et al., 2019; Prata et al., 2021). The inclusion of controls and blanks are considered a key component for most international and national environmental motioning programs (ANZG, 2018; EPA, 2016; OECD, 2013), and are underutilised in many previous microplastic studies.

Ideally, controls should be used at every step of sample manipulation, from sample collection to analysis, to comprehensively encapsulate potential extraneous contaminants. Controls typically used include laboratory controls and field controls, which can detect contamination from sample handling, dust and other atmospheric fallout. Container controls determine the level of contamination originating from the storage container. Equipment controls measure contamination introduced through contact with sampling equipment. Transport controls account for contamination of samples during transport and storage. Procedural controls mimic the sample processing workflow by applying a plastic-free matrix or reference substance (i.e., spiking) (DES, 2018). At the very least, environmental microplastic analyses should include an appropriate number of procedural controls, as even in clean environments, extraneous microplastics in field and laboratory settings are ubiquitous but extremely variable. As such it is exceptionally difficult to eliminate microplastic contamination during sample collection and processing (Gwinnett and Miller, 2021; Horton et al., 2021). Data generated from controls should be collated alongside their corresponding samples and used to correct for sample contamination. Hence, a robust correction method is needed to account for the variability of microplastics and improve accuracy of data reporting.

Although data correction is well established for many other environmental contaminants (ANZG, 2018; EPA, 2003; Li et al., 2019), in terms of microplastic samples, correction is complicated by the heterogenous suite of particles which fall under the label of 'microplastics'. Microplastics range in size by four to five orders of magnitude (0.1–5000 μm, or 1–5000 μm, depending on the lower size limit used). The shape and surface of secondary microplastics are derived from physical abrasion, and chemical and biological weathering, resulting in unique particle shapes, colours, sizes, and surface morphology (Alimi et al., 2022; Corcoran, 2020; Dawson et al., 2018; Naik et al., 2020). Further, the exact chemical composition, in terms of polymer backbone and associated additives, is typically unique to each manufacturer (Heinrich et al., 2020). Adding to this complexity, are the potential effects of the extraction process itself, which can often deform or degrade microplastic particles further (Enders et al., 2017; Gulizia et al., 2022; Kuhn et al., 2017; Munno et al., 2018; Santana et al., 2022). The resulting isolates are an ill-defined mixture of morphologically and chemically diverse particles, which are then typically grouped into categories, i.e., size, shape and polymer (Cowger et al., 2020; Kooi and Koelmans, 2019; Rochman et al., 2019), to facilitate analysis, interpretation and reporting.

Prior to carrying out this study, a mini review of 30 papers published throughout 2020 reporting on the quantification of environmental microplastics was undertaken, which revealed no consensus on how the data generated from controls were used to correct the sample data (See SI 2). Moreover, many studies still lack any kind of contamination control (e.g., Amrutha and Warrier, 2020; Chai et al., 2020; Kedzierski et al., 2020; van den Berg et al., 2020; Zhang et al., 2020). Often studies which attempted to correct for control contamination unfortunately lack adequate information for further scrutiny (e.g., Scherer et al., 2020). Overall, the most common data correction strategies employed in microplastics studies include: a) No correction – data is not adjusted irrespective of controls being implemented (e.g. Deng et al., 2020); b) Subtraction – the total number of microplastics observed in controls subtracted from the total number of microplastics in samples (e.g. González-Pleiter et al., 2020); c) Subtraction of mean – the mean microplastics value observed in controls subtracted from the mean microplastic value of samples (e.g. Lindeque et al., 2020); d) Spectral Similarity – Individual items removed based on spectral and visual matches (Kroon et al., 2018); or e) LOD/LOQ – Limits of detection (LOD) or Limits of quantification (LOQ) compared to the mean sample value (e.g. Johnson et al., 2020). Variations derived from these 5 core methods are also possible, i.e., the subtraction method (b) can be modified to include adjustments based on: total number of particles, total particles per polymer type, total particles per size range, total particles per shape class, and permutations of these, i.e., total particles per polymer AND shape, total particles per shape AND size, total particles per polymer AND size, total particles per polymer AND shape AND size, etc.

In addition to manual data correction methods, some studies have employed statistical analysis to compare between samples and control (Akoueson et al., 2020). These methods typically rely on comparison of the mean abundance of particles within the samples and controls.

This study evaluated 49 different data correction strategies based on the 5 approaches listed above (a-e) to determine the suitability of each for the application to microplastic datasets. In addition to these data correction methods, two statistical analysis methods, designed to differentiate between microplastic items in the control and samples, were also examined. Hence, a total of 51 methods were examined. Control adjustment methods assessed here were either taken from the literature and used as described or systematically modified to address method shortcomings. All methods were applied to a dummy dataset to assess their robustness, precision, and accuracy. Successful methods were then applied to real environmental datasets as a proof of concept.

2. Methods

2.1. Dummy samples

A dummy dataset was created to mimic the laboratory contamination which may arise throughout sample processing and handling. These comprised of 10 identical dummy samples which contained potassium hydroxide (KOH) digestion solution and MilliQ water as the surrogate sample matrix, i.e., no real 'sample' matrix was present. Thus, any microplastics particles isolated from the dummy samples solely originated through laboratory contamination. Dummy samples were created using precleaned glass Schott bottles (n = 10) which were placed in a clean laminar flow cabinet, to which 20 mL of 10 % prefiltered (0.45 μm PTFE hydrophilic Millipore) aqueous KOH, dispensed from a sealed bottletop dispenser, and 30 mL of MilliQ water, dispensed with a precleaned glass measuring cylinder, were added to each bottle. Bottles were capped with red polybutylene terephthalate (PBTP) Teflon-lined lids and removed from the laminar flow cabinet (ECO Heraguard Biosafety cabinet, Thermo Fisher) for filtration. The PBTP lids were

approximately 1 year old and visually appeared to be in good condition before use. The Teflon liner separated the bottle contents from the outer PBTP material. The contents of each bottle were individually filtered onto 10 precleaned 26 μm stainless-steel mesh filters using a new precleaned filtration system for each sample. The filtration system consisted of one stainless steel funnel, one stainless steel spacer, one stainless steel base and 2 blue silicone O-rings (Schlawinsky et al., 2022). As each sample was filtered, the Schott bottle, funnel and filter retentate were all rinsed 3 times with MilliQ water followed by 70 % aqueous prefiltered (0.45 μm) ethanol. Both liquids were dispensed from Teflon wash bottles (ThermoScientific). Stainless-steel filters were immediately secured in aluminium filter holders and placed into a glass Petri dish to dry in a desiccator. Filters were then covered with a glass coverslip and stored until spectral analysis. QA/QC methods employed to avoid background contamination are further outlined in the Supplementary materials.

2.1.1. Microscopy and spectral identification

Samples were examined microscopically using a Leica M205C for putative microplastics. Putative microplastics were photographed, then analysed on a PerkinElmer Spectrum 100 ATR-FTIR as per Kroon et al. (2018). Items were individually transferred to the FTIR for analysis. In some instances, items could not be analysed, i.e., too small to transfer (<100 μm) or were lost during transfer, and were disregarded. Spectra were acquired between 650 and 4000 cm^{-1}, with 16 accumulated scans at a nominal resolution of 4 cm^{-1}, and background scans, with no sample present, were acquired every tenth sample. The raw spectra were processed with the standard PerkinElmer Spectrum Data tune-up function and included Beer-Norton strong apodization and automatic baseline correction. The atmospheric water/CO_2 region between 2500 and 1900 cm^{-1} was excluded when comparing to spectral libraries. Polymer identification were performed using the siMPle Library (Primpke et al., 2020a) and the commercial NICODOM library (Polymers and Additives, Coatings, Fibres, Dyes and Pigments, Petrochemicals; NICODOM Ltd., Czech Republic). Spectral library matches were considered reliable > 70 %. Spectra with 70–60 % match were subjected to additional manual examination and assigned as per Kroon et al. (2018). In instances where the signal quality was poor or inconsistent with the hit, the match was disregarded. Due to the abundance of red PBTP particles in the dummy samples, a subsampling FTIR protocol was designed. All items visually identified as red PBTP were photographed, thereafter, 25 % or 5 items per filter, whichever was higher, were randomly selected using a random number generator and analysed by FTIR. Where all analysed items returned the same spectral signature, all putative items having the same visual characteristics were assigned the same polymer identity. If the spectra of the subsampled items differed, 50 % or 10 items per filter, whichever was higher, of the subsampled items were analysed by FTIR, and the photographs re-examined. Where more than 2 differing spectra occurred within the remaining subsampled items (50 % or 10 items), all items were analysed. The physicochemical characteristics of shape, colour, size (length) and chemical (polymer) identity were recorded for each particle.

2.1.2. Defining physicochemical variables

For the Shape variable, particles were assigned to one of two categories: 'Fibre' or 'Fragment'. A fibre was defined as a particle with length to width ratio > 3. A fragment was defined as a particle with length to width ratio ≤ 3 (Vianello et al., 2019). The length of each particle was measured using FIJI (Schindelin et al., 2012) and calculated as the Feret Diameter (the longest distance between any two points along the selection boundary). Fibres were measured down the midline using the segmented line tool. To define categorical variables for Size, the length of each item was assigned to one of four size groupings. These four groups were selected based on the interquartile range (25 %, 50 %, 75 %, 100 %) of the length data, due to the lack of standardized grouping system for the microplastic literature (Ziajahromi and Leusch, 2022). Here, size was categorised as Group 1: < 158.3 μm; Group 2: 158.3–246.9 μm; Group 3: 246.9–548 μm; Group 4: >548 μm. Polymer was assigned based on FTIR spectra to one of 12 categories. Four categories corresponded to polymers of natural origin: cellulose (i.e., cotton), rayon, keratin (i.e., wool or fur) or cellulose:keratin, the other eight to synthetic polymers: polypropylene (PP), polyproplyene:polyethylene (PP:PE), cellulose:PP, acrylic, PBTP, polyester (PES), polytetrafluoroethylene (PTFE) and alkyd (synthetic paint). Although cellulose and keratin are not microplastics, their conversion into made-made products means they are typically considered microdebris and anthropogenic in origin, and hence are often reported when detected in microplastic samples. As such, they are included in this study. Colour was used only as a secondary characteristic, i.e., defined by eye and recorded for each particle as: black, blue, brown, green, grey, orange, purple, red, or transparent/white. The Limit of Detection (LOD) was defined as mean + 3X the standard deviation (SD). The Limit of Quantification (LOQ) was defined as mean + 10X SD (Brate et al., 2018).

2.2. Data correction methods

The particles extracted from the dummy dataset were used to explore different mathematical methods to adjust microplastic datasets. Correction methods applied here are outlined in Table 1, and a brief

Table 1
Data adjustment methods and variations on the core method (denoted by #). Combination methods are based on core methods combined with Spectral Similarity. Explanation of each method and the calculation is given in the SI.

Types of Adjustments	Method Description	Variations on the Core Method	Method number	Combined Method number
No correction	Results are not adjusted	None		
Subtraction	Total number observed in controls subtracted from total number in samples	• Total • Polymer • Size • Shape • Polymer*Shape • Shape *Size • Polymer *Size • Polymer*Shape *Size	1# 2 3 4 5 6 7 8	34 35 36 37
Subtraction of mean	Mean value observed in controls subtracted from the mean value of samples	• Total • Polymer • Size • Shape • Polymer*Shape • Shape *Size • Polymer *Size • Polymer*Shape *Size	9# 10 11 12 13 14 15 16	38 39 40 41
Subtraction of mean from individual samples	Mean value observed in controls subtracted from each induvial sample values	• Total • Polymer • Size • Shape • Polymer*Shape • Shape *Size • Polymer *Size • Polymer*Shape *Size	17# 18 19 20 21 22 23 24	42 43 44 45
Subtraction of LOD/LOQ	LOD or LOQ subtracted from the total value of each sample	• Total • Polymer • Size • Shape • Polymer*Shape • Shape *Size • Polymer *Size • Polymer*Shape *Size	25# 26 27 28 29 30 31 32	46 47 48 49
Spectral Similarity	Individual items removed based on spectral and visual match	None	33#	

explanation of each method and associated formulas is given in the Supplementary materials 1.3.

The 10 dummy data replicates were randomly assigned as either 'Control' (n = 3 replicates) or 'Sample' (n = 7 replicates) treatments. Particles isolated from the control replicates were used to adjust the number of particles isolated from the sample replicates, resulting in an adjusted 'MP Sample^{-1}' or 'Total MPs' value. The remaining particles after correction were then expressed as a percent of the original unadjusted data. This process was repeated 12 times using different random assignments of 'Control' and 'Sample' replicates (Table S1) to ensure the results were robust and repeatable. As this study used identical dummy samples for control and sample treatments, all isolated particles originated from laboratory sources throughout the sample processing. Thus, ideally after correction the adjusted value for MP Sample^{-1} should be zero, or close to zero. A correction method was deemed successful if it managed to remove an average of 95 % of the original data across the 12 randomisations. All methods that satisfied the average 95 % threshold were then applied to three environmental datasets from previously published work; they include Great Barrier Reef sediments (Santana, 2022), benthic filter feeding Saucer scallops (Dawson et al., 2022), and fish muscle tissue purchased from local seafood shops for human consumption (Dawson et al., 2022). For the LOD/LOQ methods, if the abundance of particles was below the LOQ, it was treated as 0.

2.2.1. Optimising the spectral similarity method

Method 33 relies on a total subtraction of items based on a spectral similarity and visual characteristics (Kroon et al., 2018). Briefly, laboratory coats and equipment that may contaminate the workspace or come into contact with the samples, along with particles found in controls, are photographed and spectrally characterised as outlined above. These potential extraneous contaminants form a visual and spectral contaminant library against which all sample items are compared both visually and spectrally. When a sample particle matches a contaminant or control library item with > 90 % spectral similarity and visual similarity (i.e., same colour, shape, texture), the particle is removed from the dataset. This correction method provides a count of total sample particles minus items confirmed to be contaminant particles.

All particles isolated from the control samples were added to a control library. Each randomisation had a unique control library, depending on which replicates were assigned as controls. However, particles in the contaminant library remained consistent across all randomisations. Items added to the contamination library were commonly used laboratory items found in the immediate vicinity of the work area and which may make contact with the samples or items used during processing. Items in the library are listed in Table S2.

Initial application of method 33 to the dummy samples returned limited matches results, therefore a more flexible spectral similarity cut-off (90 %, 85 %, and 80 %), for contaminant and control library comparisons, was assessed. The method was optimised using the 1st randomisation of the dummy samples, where the new threshold was selected based on the cut-off which removed the highest number of particles, without compromising on data quality and increasing erroneous matches. Erroneous matches were defined as items that matched spectrally, as well as being of the same generic colour and shape class, but visually were not considered a valid match (e.g., Fig. S1). As the dummy samples contained 10 % potassium hydroxide, which is a known bleaching agent for cellulose-based polymers (Dawson et al., 2020), transparent coloured cellulose-based items were considered a match even if they matched to coloured cellulose-based items. A one-way ANOVA with Tukey post hoc test was used to compare the method 33 optimisation cut-offs. Analysis was carried out using GraphPad Prism 8.4 or R version (4.1.2) and RStudio (2022.02.0).

2.3. Control analysis methods using statistical analysis

In addition to the methods outlined above, which manually adjust sample data based on control data, some studies have employed statistical analysis methods to compare between samples and controls (Akoueson et al., 2020; Rasta et al., 2021; Su et al., 2019). These used a statistical approach to determine if the concentration in the sample data was significantly greater than the controls, and therefore reliable for reporting. Here, two statistical analysis methods were applied to the dummy sample data (Table 2). These were a t-test, and a Bayesian regression model. While one t-test was conducted per sample randomization (i.e., 12 t-tests were conducted in total), Bayesian regression analysis was only conducted once (i.e., without using the 12 randomizations) as an exploratory exercise to facilitate a preliminary discussion on the applicability of this statistical approach to microplastics QA/QC. For Bayesian regression analysis, Schott bottles labelled 1–5 were categorised as controls, while those labelled 6–10 were the samples.

2.3.1. T-test control analysis

T-tests have previously been used to differentiate between the microplastic abundance in controls and samples (e.g. Su et al., 2019). In the present study, normally distributed data was analysed using an independent Welch's t-test and non-normal data was analysed using a Mann-Whitney test. Normality was assessed using Shapiro-Wilk normality test and a $p\ value > 0.05$ indicated that the microplastic abundance in the samples was identical to the controls. Analysis was carried out using GraphPad Prism 8.4 or R version (4.1.2) and RStudio (2022.02.0).

2.3.2. Bayesian regression analysis

Bayesian regression analysis has not been applied in the microplastic literature for QA/QC purposes thus far. The Bayesian framework allowed for the direct calculation of posterior differences (mean difference) of microplastic shape, size, colour, and polymer between samples and controls, as well as its associated uncertainty. Because posterior distributions are probabilistic measures of a parameter value, one can also quantify, for example, the probability of a particular difference occurring.

Based on a series of preliminary explorations on the model yield of best fit and residuals (not shown), shape and size were deemed to interact and were therefore analysed together. Specifically, a model with a gamma distribution and log link was used to assess differences between shape and size (Model 1, Eq. 1), while models with categorial distribution and logit link were used to assess polymer and colour (Models 2 and 3, Eqs. 2 and 3). Control and sample are represented as "type" in the Bayesian regression models. Models included shape, colour and polymer as fixed factors. For Model 1 specifically, samples were included as random effect given that each sample contained multiple microplastics. Furthermore, in this model, one item > 4000 μm was considered an outlier and excluded from the dataset.

$$\text{size} \sim 0 + \text{Intercept} + \text{type} * \text{shape} + (1|\text{dummy\_sample}) \quad (1)$$

$$\text{polymer} \sim 0 + \text{Intercept} + \text{type} \quad (2)$$

$$\text{colour} \sim 0 + \text{Intercept} + \text{type} \quad (3)$$

Numerical simulations were conducted using four chains (Models

Table 2
Statistical analysis methods.

Statistical method	Method Description	Method Number
Independent t-test	Mean abundance within the samples is compared to the mean abundance of the controls	50
Bayesian regression model	Posterior differences (mean difference) of microplastic shape, size, colour, and polymer, and colour between samples and controls, is modelled, as well as associated uncertainty.	51

1–3), 4 or 1 cores (Model 1 and Models 2 and 3, respectively) and 10,000 iterations (models 1–3). Normal priors with a mean of 0 and SD of 2 were used for the population-level effects. Gamma priors were set with a location of 2 and inverse scale of 1 for the group-level SD and shape parameter. Model validation and analysis were conducted using the brms package (Bürkner, 2017). Influence of priors in the posterior distribution, as well as chain convergence and residuals were assessed for each model to validate whether these were in adequate fit to the data (Model 1: Figs. S2–5; Model 2: Figs. S6–7, Model 3: Fig. S8–9). Posterior predictive checks were used to investigate how well the models replicate the overall distribution of the data. As DHARMa residuals do not work with categorical models, the validation steps for Eqs. 2 and 3 considered posterior predictive checks only. Differences between control and sample were assessed using the R package 'emmeans' (version 1.6.0) and posterior distribution of differences expressed as fold change (i.e., ratios) between control and samples. Thus, differences between both treatments were expected to be centred around 1, while the 95 % credible intervals were expected to overlap 1, with 1 representing no difference between control and sample treatments.

2.4. Environmental datasets

To validate the applicability of the methods to abiotic and biotic microplastic data, those methods that successfully removed 95 % of the original data in the dummy samples, as well as the statistical analysis methods, were applied to the three previously published environmental datasets, chosen based on access to complete raw datasets, including spectral files and custom analysis-specific contamination libraries. For all three datasets, the size grouping cut-offs were based on the interquartile range for each variable.

Dataset 1: Previously, 10 wild caught Ballot's Saucer Scallops (*Ylistrum balloti*) were subdivided into muscle and gastrointestinal tissues and analysed for microplastics inside a clean air cabinet (Biosafety class 2), as described in (Dawson et al., 2022). Procedural negative controls and blanks, prepared with each extraction batch, were conducted in parallel to the sample processing. Procedural controls consisted of digestive solution (10 % KOH, MilliQ water and 80 % ethanol). Four blanks of MilliQ water were placed in each corner of the workspace whist the samples were being dissected. All controls/blanks were filtered onto individual stainless-steel filters. This resulted in 20 samples and 21 controls/blanks for this dataset. QA/QC and contamination protocols are outlined in Dawson et al. (2022).

Dataset 2: Previously, three barramundi (*Lates calcifer*) muscle fillets, prepared and purchased from retail shops in Townsville, Australia, were analysed for microplastics (Dawson et al., 2022). Procedural negative controls and blanks followed the same protocol as for Dataset 1. This resulted in 3 samples and 5 controls and blanks for this dataset. QA/QC and contamination protocols are outlined in Dawson et al. (2022).

Dataset 3: Previously, 10 sediment samples from Lizard Island coral reefs were collected and analysed for microplastics (Santana, 2022). Four MilliQ blanks were prepared for each extraction batch and placed in each corner of the workspace whist the samples were being processed. Blanks were only uncovered when samples were also exposed to air. The four blanks used per batch were filtered into one stainless-steel filter. This resulted in 10 samples and 2 blanks for this dataset. When possible, work was conducted inside a fume hood. QA/QC and contamination protocols are outlined in Santana (2022).

3. Results and discussion

3.1. Dummy sample characterisation

Despite all dummy samples being identical in terms of matrix and handling, the abundance, shape, size, colour and chemical composition of contaminant particles was not consistent across the 10 replicate bottles (Fig. 1). Between 4 and 20 particles were isolated from each bottle (Fig. 1a). Polymer distribution likewise varied across all bottles (presented from most common to least common polymer± SD: 4.7 ± 1.8

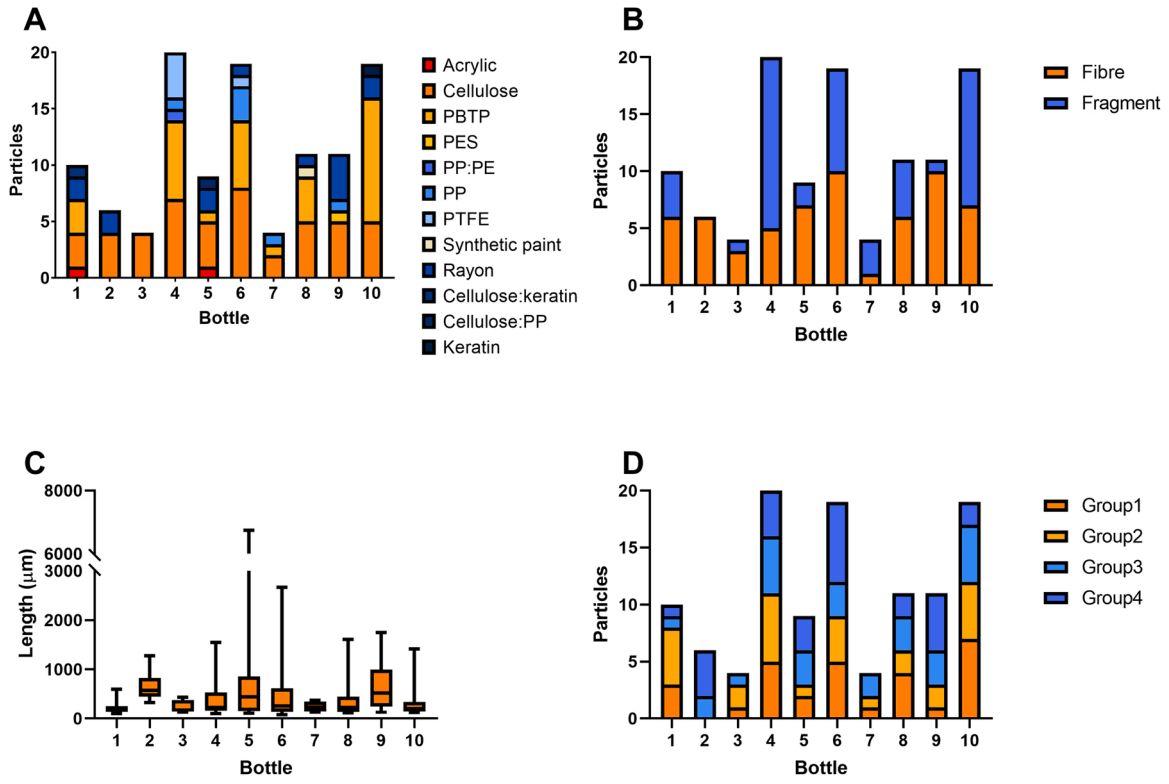

Fig. 1. Microplastic composition in the 10 dummy sample bottles described by a) polymer identity and abundance, b) abundance of fibres and fragments, c) length (size) of each particle, and d) abundance of particles in each size group.

cellulose, 3.3 ± 3.7 PBTP, 1.4 ± 1.3 rayon, 0.6 ± 1.0 PP, 0.5 ± 1.3 PTFE, 0.2 ± 0.4 acrylic, 0.1 ± 0.3 PES, 0.1 ± 0.3 PP:PE, 0.1 ± 0.3 synthetic paint, 0.1 ± 0.3 cellulose:keratin, 0.1 ± 0.3 cellulose:PP, 0.1 ± 0.3 keratin). Cellulose was the only polymer consistently isolated from all 10 bottles. Six of the 12 polymer types were only isolated from a single bottle each. Particle shape also varied across the 10 bottles (Fig. 1b), although most bottles contained both fibres and fragments (6.1 ± 2.8 fibres, 5.2 ± 5.1 fragments). Regarding size, even particles of the same polymer composition, within the same bottle varied in length (Fig. 1c, Fig. S10). Mean particle size was 545.8 ± 924.5 μm. Some contaminating particles were immediately recognisable as originating from laboratory items, i.e., red PBTP fragments from the Schott bottle lids, green and bleached transparent cellulosic fibres from green-dyed 100 % cotton laboratory coats, yet for the majority, a source could not be confirmed.

3.2. Optimisation of the Spectral Similarity Method

The spectral similarity threshold for Method 33 was examined for 90 % (as arbitrarily set by Kroon et al., 2018), 85 % and 80 %. None of the three cut-offs (90, 85, or 80 %), yielded erroneous matches, although items assigned as a cellulose polymer often strongly matched to contaminant items assigned as rayon (i.e., regenerated cellulose) based on the NICODOM library match, and vice versa. As it is sometimes difficult to differentiate between modified or regenerated cellulose and cellulose using FTIR spectra (Comnea-Stancu et al., 2016), and taking a conservative approach, these items, providing they were visually similar, were also considered a match. The 85 % threshold did not significantly increase the number of matches ($p > 0.05$), however, the 80 % threshold returned significantly more matches to the contaminant library than the 90 % threshold ($p = 0.0095$) (Table S3). Thus, to increase the total number of matches, the 80 % threshold was selected as the spectral threshold for Method 33.

3.3. Control adjustment methods

3.3.1. Uncorrected data (no correction)

The unadjusted particle concentration of samples in each of the 12 randomisations is shown in Fig. 2. Before correction, the total number of microplastics across all samples ranged between 55 and 99 (7.86 and 14.14 MP Sample^{-1}). This range varied considerably, depending on which bottles were assigned as samples and controls. For example, the lowest abundance was seen in randomisation 12, where the three bottles containing the most particles were assigned as controls. The opposite was true of randomisation 11, where the three bottles with the least number of particles were assigned as controls. However, there was no significant difference between the particle abundance across the randomisations (Kruskal-Wallis, $p > 0.05$).

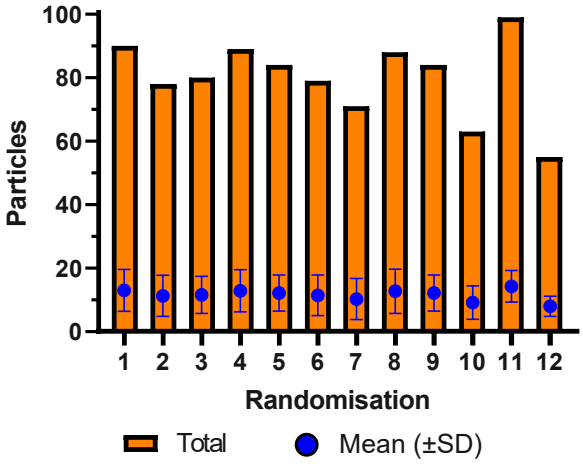

Fig. 2. Unadjusted particle abundance (orange bars) and mean ± SD (MP sample^{-1}) (blue circles) concentration for particles isolated from the 7 sample bottles in each of the 12 randomisations.

3.3.2. Total subtraction method

Application of the Total Subtraction method (Method 1) removed <50 % of the original data (Figurea); this was consistent for all method variations (2,3,4,5,6,7,8). Overall, these methods did not account for the heterogeneity amongst the isolated particles. For example, the PBTP Schott lids used in this experiment were all the same polymer, shape, and colour, as well being the same age with similar usage. However, the number of red PBTP fragments varied considerably across the 10 bottles, thus even after applying the adjustment methods, numerous PBTP particles remained in the final total. Thus, using the total subtraction methods these were considered to be 'real' sample microplastics rather than laboratory contamination. The total subtraction methods were deemed unsuitable and were not applied to the environmental datasets.

3.3.3. Average subtraction method

The Average Subtraction methods (9,10,11,12,13,14,15,16) performed better overall than the Total Subtraction methods (Fig. 3). Most of these methods successfully removed >50 % of the original particle data, however, there was high variability amongst the 12 randomisations. For example, after applying Method 9, 18.40 % ± 22.03 of particles remained. None of the methods satisfied the mean 95 % threshold, and thus were not applied to the environmental datasets.

3.3.4. Average subtraction per individual samples method

The Subtraction of the control average from individual samples methods (17,18,19,20,21,22,23,24) were not as effective at removing contamination data as the Average Subtraction method. Although the core Method (17) was able to remove >50 % of the original data, with 27.77 ± 19.30 % remaining, none of the other variations (18,19,20,21,22,23,24) were as effective (Fig. 3a). None of these methods were applied to the environmental datasets.

3.3.5. Limits of detection and limits of quantification (LOD/LOQ) method

All the LOD/LOQ methods (25,26,27,28,29,30,31,32) successfully removed at least 50 % of the original data after application. Based on means, Methods 25, 27, and 28 removed >95 % of the data, however, the SD of Method 25 was more than double the mean (1.32 ± 4.58) (Fig. 3a). Methods 25, 27 and 28 were applied to the environmental datasets for validation (Fig. 3b).

3.3.6. Spectral similarity method

Application of the Spectral Similarity method using the optimised threshold of 80 % resulted in 33.25 % ± 4.41 of the original data remaining after the correction (Fig. 3a). However, the variation within the 12 randomisations was very small (4.41 SD), suggesting that despite not removing 95 % of the data, this method is repeatable. This method relies on consistent polymer purity to produce a high quality spectrum (e.g., without interference of polymer changes induced through digestion or surface coating by foreign materials), which when analysing environmental microplastics may not be achievable due to, for example, degradation, small irregular size, surface biofouling, digestion or isolation methods (Kedzierski et al., 2019; Masry et al., 2021; Zvekic et al., 2022). As such this method was deemed unsuitable for analysis of the environmental datasets.

3.3.7. Combination methods

All the spectral similarity combination methods were successful at removing at least 50 % of the particle data (34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49) (Fig. 3a). However, only methods 46, 48 and 49 removed 95 % of the data. Similar to Method 25, Method 48 was variable (3.72 ± 3.41). Methods 46, 48 and

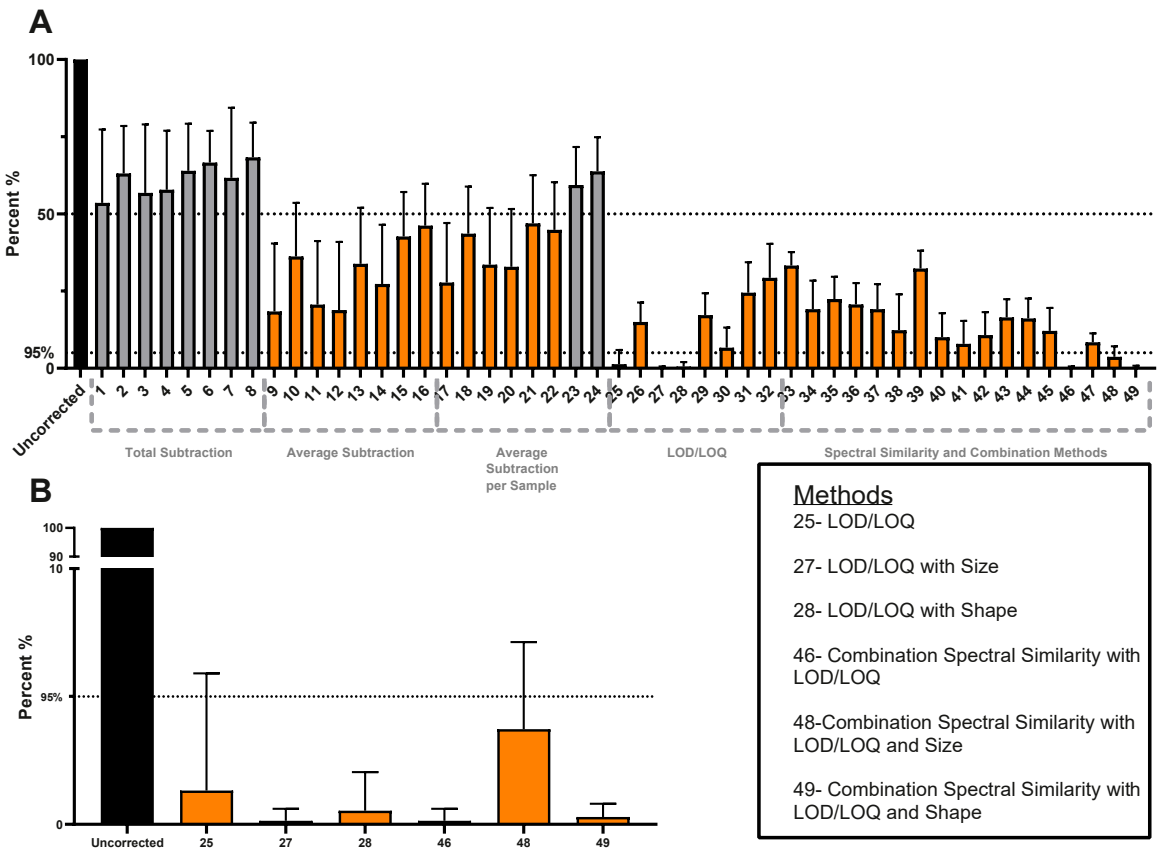

Fig. 3. The percent (mean and SD) of data remaining after each correction method was applied. Orange data indicates methods which removed at least 50 % of the original data. A) all 49 non-statistical methods, B) methods that successfully removed 95 % of the original data (<5 % remaining). See Table 1 for a description all methods.

49 progressed to validation with the environmental datasets (Fig. 3b).

3.3.8. *Methods satisfying the 95 % threshold*

Overall, only 6 of the 49 non-statistical methods successfully removed an average of 95 % of the original data from the samples (25, 27, 28, 46, 48, 49) (Fig. 3b). These methods were all based on the LOD/LOQ core method (25) or the LOD/LOQ method combined with Spectral Similarity (46). Shape (28,49) or size (27,48) were the only variations of the two core methods that retained 95 % efficacy.

LOD and LOQ are used to describe the lowest concentration that can be reliably measured by an analytical procedure (Armbruster and Pry, 2008). The advantage of using the spectral similarity combination method is that the LOD is reduced by eliminating known contaminants from the samples and controls, thus only the unexpected items remain. The LOD then gives confidence for the likelihood of detecting 'real' data above the variable background noise. Deducting known microplastic contaminants using LOD or LOQ has been successfully utilised in several previous studies (Brate et al., 2018; Gwinnett and Miller, 2021; Johnson et al., 2020; Rødland et al., 2020). For example, Gwinnett and Miller (2021) used a similar technique to subtract known microplastic items from seawater samples collected under stringent and relaxed QA/QC protocols.

Most of the unsuccessful methods tested here relied on the assumption that contaminating particles were identical and consistent across both the samples and controls, in terms of polymer, shape, size and abundance. This assumption is valid when analysing most contaminants, especially if these contaminants are chemically dissolved and homogenously distributed within the sample, e.g., methylmercury (e.g. Hong et al., 2012). However, microplastics are complicated as they are composed of a suite of chemicals in particulate form, with various physical characterisations (Rochman et al., 2019). The dummy samples in this study clearly demonstrate that this assumption is not applicable to contamination of microplastics within a laboratory environment. In this study, even traceable items such as the PBTP and PTFE displayed considerable variability in abundance and chemical characteristics across the samples. This disparity between microplastics within the controls and samples, or even within the controls has been reported in several other studies (Athey et al., 2020; Belontz and Corcoran, 2021; Klein and Fischer, 2019; Su et al., 2019). For example, Athey et al. (2020) used two procedural controls when analysing wastewater effluent and isolated 19 and 56 microplastics from the two replicates, respectively. Klein and Fischer (2019) reported fibres comprised 51 % of the total particles isolated from the controls whereas fibres only comprised 5 % of the total particles within the samples. Belontz and Corcoran (2021) identified contaminant fibres ranging in size from 870 μm to 4320 μm across the controls which consisted of multiple colours and polymers. Even studies which did not rely on visual detection methods, demonstrated this variability. Duplicate measurement of microplastics in human blood, quantified using GC-MS, also displayed variability in the concentration of each polymer between the replicates (Leslie et al., 2022).

3.3.9. *Optimal number of variable categories*

Across all methods, the number of categorical groups (n = 18; Shape = 2, Size = 4 and Polymer = 12) defined within each physicochemical variable appeared to influence the amount of data able to be removed when compared to the core method. As more categorical groups are added into a core method, i.e., where methods combined variables, these were additive, with less data removed, and the thus the method becomes less reliable. For example, the Average Subtraction Method 14

applied the variables of Shape AND Size, incorporating eight (2*4, respectively) categorical groups (resulting in 27.31 ± 19.20 % of data remaining), while Method 16 considered Shape AND Size AND Polymer, to give 96 categorical groups (2 *4 *12, respectively) (with 46.18 ± 13.56 % data remaining). This trend was observed for all core methods, but was most apparent in the LOD/LOQ Methods 29–32 (method 29 had 24 categorical groups resulting in 17.19 ± 7.10 % remaining; method 30 had 8 categorical groups resulting in 6.68 ± 6.53 %; method 31 had 48 categorical groups resulting in 24.47 ± 9.85 %; method 32 had 96 categorical groups resulting in 29.28 ± 11.04 % data remaining).

Manipulation of the variable Size, where particles were re-assigned to one of either 2, 4, 6, 8, or 12 categorical groups (Table S4), enabled the determination of the optimal number of categorical groups which should be employed in a correction method (see Supplementary Material 1.3). Applying this approach to the LOD/LOQ method established that to adhere to the 95 % threshold, at most only 2–5 categorical groups can be included in the core method (Fig. 4), beyond this, the data become extremely variable. This suggests that, for the core methods explored here (1,9,17,25), the variable Polymer, with an almost infinite number of potential commercial polymers detectable, is unlikely to be a useful variable to include, with possible exception in cases where a dataset is reasonably homogenous (i.e., <5 polymers detected). Overall, as more variables are added to the analysis the more 'specific' the method becomes, and thus, more items are detected above the background level. In other words, the more specific the method is, the less a method can cope with the heterogeneity of the isolated contamination particles, and thus more contamination particles are assumed to be real sample particles. Conversely, more 'general' methods were more inclusive and assumed more items are contaminants, with the caveat that whichever method is chosen, there is always a trade-off between data resolution and method specificity.

3.4. Statistical analysis methods

3.4.1. T-test control analysis

Two sample t-tests using mean microplastic abundance found no statistical difference ($p > 0.05$) between controls and samples in 10 of the 12 randomisations (Table S5, Fig. S11). One randomisation found the control mean to be significantly higher than the sample mean ($p < 0.001$). The final randomisation found the opposite, where the sample mean was significantly higher than the control mean ($p < 0.01$). Thus, the abundance of microplastics in the samples was found to be significantly higher than that of the control in only one test ($p < 0.001$).

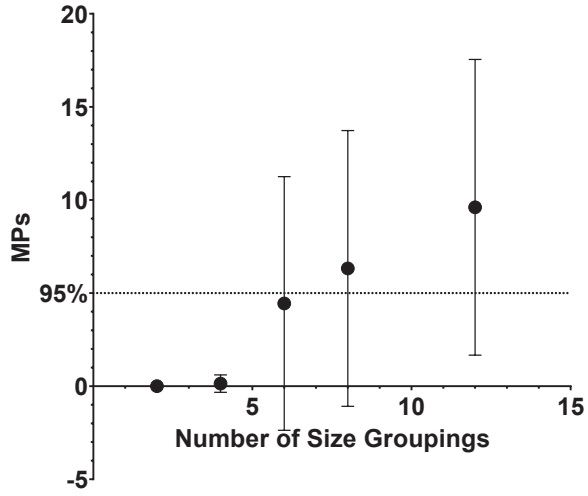

Fig. 4. The amount of microplastics (MPs) remaining after applying the LOD/LOQ method with 2, 4, 6, 8 or 12 size groupings.

Overall, in terms of simplicity, *t*-tests required little data manipulation, and correctly determined the particles in the sample to be non-distinct from background contaminant in 92 % of tests. Therefore, this method was also applied to the environmental datasets, although it proved slightly less reliable then the LOD/LOQ methods. This may be due to the small number of control (n = 3) combined with the large variation in microplastic content, leading to large random variations. The adding additional control replicates is expected to improve the accuracy of this background correction method.

3.4.2. Bayesian regression analysis

The number of particles in the 5 bottles were assigned as controls and 5 bottles assigned as samples are given in Table S6, and the distribution of particles was fairly even (Fig. S12). Summaries from each Bayesian regression model are presented in the Supplementary materials (Tables S7-S9). Model 1, which analysed differences in length based on shape, estimated a 0.65-fold decrease in the average size of fibres between control and sample treatments. However, the 95 % credible intervals overlapped 1, suggesting lack of strong evidence for the observed differences in size (Table S19, Fig. 5). For fragments, the average size was similar between controls and samples (i.e., average fold change of 1), and the 95 % credible intervals overlapped 1. Overall, results from Model 1 indicate microplastic fibres and fragments had similar sizes between controls and samples, which was expected as, theoretically, all particles within the bottles originated from common laboratory items.

Bayesian regression Model 2 indicated most polymer types were present at different proportions within controls and samples. Only cellulose and rayon had proportions close to 1 and had 95 % credible intervals overlapping 1, suggesting similarities between the sample and controls (Table S11, Fig. 6). For the remining 10 polymers, a larger sample size would be required to reduce the uncertainty of the model output. Formal power analysis could be used to estimate the sample size required to incorporate polymer type into Bayesian QA/QC methods for microplastics. Yet, given the unpredictable number of microplastics present in field samples, calculating the number of microplastics necessary to use polymer as a category for contamination control strategies seems a fruitless endeavour.

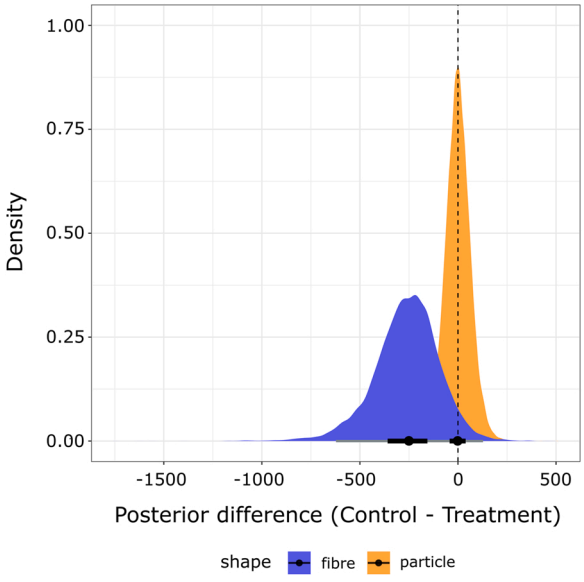

Fig. 5. Bayesian posterior distribution of differences between control and samples in terms of microplastic length for fibre or fragment. Black point represents the mean difference. Horizontal error bars are Bayesian 50 % (thick) and 90 % (thin) credible intervals. Absolute differences between both treatments were expected to be centred around 0, which is indicated by the dashed black line. See Table S10.

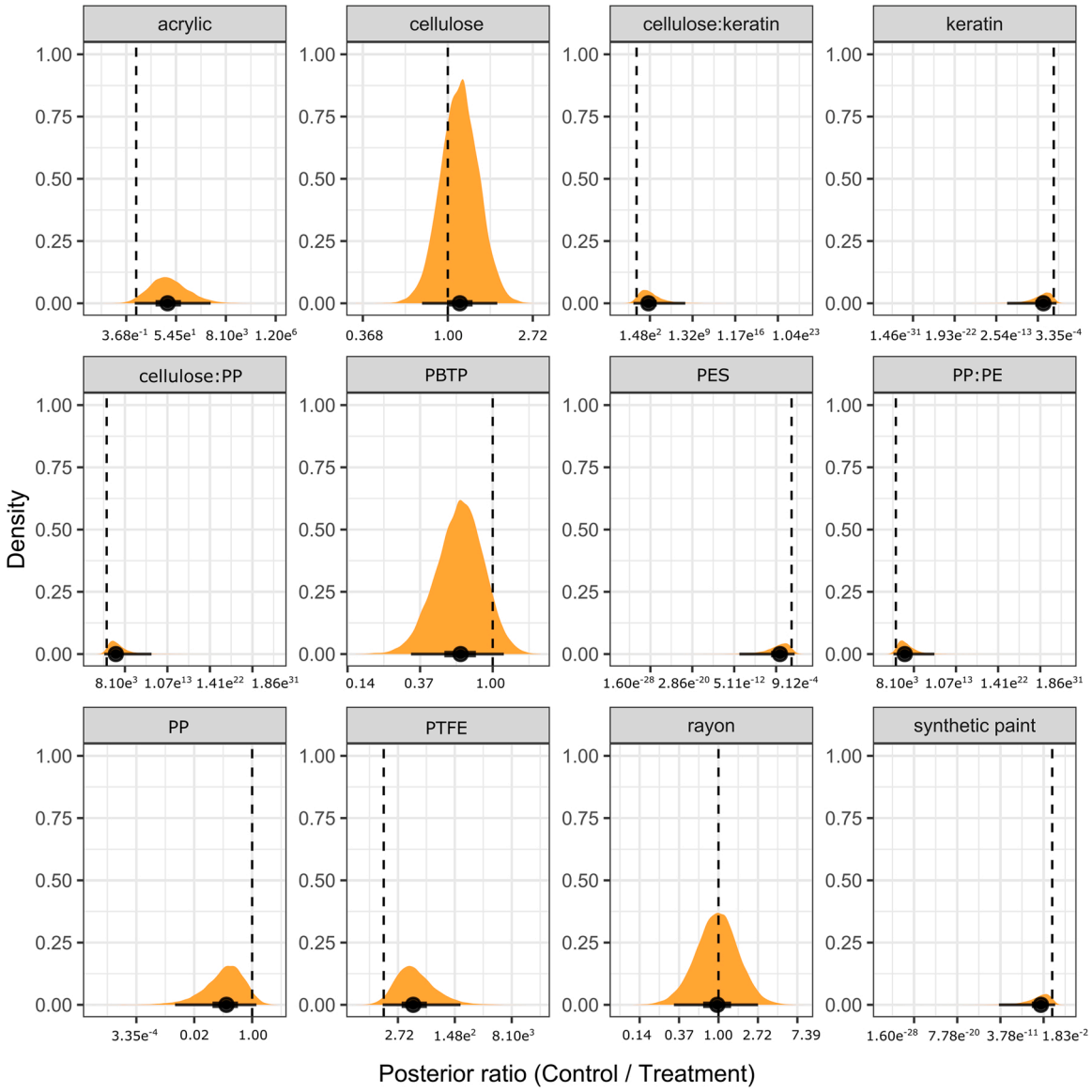

Fig. 6. Bayesian posterior distribution as the ratio between control and samples per polymer type. Black point represents the mean difference. Horizontal error bars are Bayesian 50 % (thick) and 90 % (thin) credible intervals. Absolute differences between both treatments were expected to be centred around 1, which is indicated by the dashed black line. See Table S10.

Lastly, Model 3 aimed to analyse similarities between the various microplastic colours in control and sample treatments. Results indicate only four out of the nine microplastic colours were found in similar proportions between sample and control treatments. These colours were green, transparent, blue, and brown (Table S12, Fig. S13). All other colours had ratios not centred around 1, despite having acceptable credible intervals. Thus, following the interpretation of Model 2 outputs, Model 3 also did not provide strong evidence for similarities between sample and control treatments based on particle colour.

Based on one simulation, the Bayesian regression approach correctly determined the sample to be non-distinct from background contamination only when microplastics were considered by Shape and Size. Whereas particles isolated from the samples were found to be distinctly different from the control when considered by Polymer or Colour. Furthermore, Bayesian regression analysis for microplastic contamination control required significant data manipulation to correctly determine differences between microplastic contents in samples and controls. Thus, this approach was deemed complex, time-consuming, and not necessarily reliable for QA/QC practices in microplastic research to correct data from contamination. Hence, the Bayesian regression approach was not applied to the environmental datasets.

3.5. *Environmental datasets*

Methods 25, 27, 28, 46, 48 and 49 were applied to three environmental datasets. The Barramundi muscle contained 202 items across the three samples, with only 6 items isolated from the five controls and blanks. After application of all correction methods, microplastics were still detected in all three samples (Fig. 5A), although when using methods that relied on the Shape variable (Method 28 and 49), two of the three samples returned concentrations of 0 MP fragments sample^{-1}, the concentration in these samples being less than the LOQ. The sediments dataset followed the same trend (Fig. 5B), with only two of the 10 samples having fragments in high enough concentrations to be quantified above the LOD/LOQ (Method 28 and 49, Tables 3 and 4). Overall, the scallops were found to have less items present in the tissue, both gastrointestinal (GIT) and muscle (15 and 21 items, respectively) than in the controls (38 items). Five of the six correction methods resulted in a

Table 3
Test datasets analysed using the six methods which satisfised the 95 % threshold criteria. MP = microplastic, LOD/LOQ = limits of detection/limits of quantification, GIT = gastrointestinal tract.

					Fibre		Fragment		Fibre		Fragment		Size1		Size2		Size3		Size4		Size1		Size2		Size3		Size4	
Matrix	Method 25		Method 46		Method 28				Method 49				Method 27								Method 48							
	LOD/ LOQ	MP sample	LOD/ LOQ	MP sample	LOD/ LOQ	MP sample	LOD/ LOQ	MP sample	LOD/ LOQ	MP sample	LOD/ LOQ	MP sample	LOD/ LOQ	MP sample	LOD/ LOQ	MP sample	LOD/ LOQ	MP sample	LOD/ LOQ	MP sample	LOD/ LOQ	MP sample	LOD/ LOQ	MP sample	LOD/ LOQ	MP sample	LOD/ LOQ	MP sample
Scallops																												
-GIT	13.11/	<LOD	7.47/	<LOD	6.26/	<LOD	9.08/	<LOD	4.55/	<LOD	4.91/	0	5.24/	0	4.89/	<LOD	3.94/	<LOQ	3.77/	<LOD	3.40/	0	3.19/	<LOD	3.58/	<LOQ	0.97/	<LOD
-Muscle	37.49	<LOD	21.56	<LOD	17.88	<LOD	26.40	<LOD	13.39	<LOD	13.39	<LOD	15.35	<LOD	14.53	<LOD	11.91	<LOD	11.43	<LOD	10.10	<LOD	9.54	<LOD	11.18	<LOQ	3.03	<LOQ
Fish	3.44/	67.33	1.87/	56.33	3.00/	60.67	2.07/	5.67	0.00/	49.67	1.87/	5.67	1.87/	16.67	1.40/	17.00	1.40/	17.00	2.80/	16.67	1.40/	16.67	1.40/	17.00	0.00/	17.00	0.00/	16.67
Fillets	8.69		5.30		8.60		5.50		0.00		5.30		5.30		4.20		4.20		8.40		4.20		4.20		0.00		0.00	
Sediments	3.00/	18.00	2.00/	15.90	1.00/	14.50	2.00/	2.00	0.00/	12.60	2.00/	1.75	4.00/	1.33	0.00/	4.5	2.00/	2.13	0.00/	4.6	2.00/	3.10	0.00/	4.50	0.00/	3.70	0.00/	3.20
	6.50		5.50		1.00		5.50		0.00		5.50		11.00		0.00		5.50		0.00		5.50		0.00		0.00		0.00	

Table 4
The number of samples in each dataset where the sample abundance was below the limits of detection (LOD) or limits of quantification (LOQ) (i.e 'nondetects'). GIT = gastrointestinal tract.

Matrix (n)						Fibre		Fragment		Fibre		Fragment		Size1		Size2		Size3		Size4		Size1		Size2		Size3		Size4	
		Method 25		Method 46		Method 28				Method 49				Method 27								Method 48							
		Number of samples below limit																											
		LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ
Scallops	GIT (10)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	9	10	10	10	10	10	10	10	9	10	10	10
	Muscle (10)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Fish Fillets	(3)	0	0	0	0	0	0	2	2	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sediments	(10)	0	0	0	0	0	0	2	8	0	0	2	8	4	9	0	0	1	8	0	0	4	9	0	0	1	8	0	0

LOD of ~4 MP sample^{-1}, although Method 25 returned a high LOD of 13.1. The reported concentration of microplastics for all the GIT (Fig. 7C) and muscle tissue (Fig. 7D) for all methods was 0 MP sample^{-1}. Based on these results, either all items isolated from the scallops originated from laboratory contamination rather than the sampling environment, or the environmental burden of items isolated from the scallops is too low to be detected when compared to the background laboratory contamination.

Overall, the six methods were easily applied to the environmental datasets, although some of the datasets were encumbered by the variability within the controls. This variability resulted in high SD around the mean for many of the methods. For example, scallops analysed using Method 46 had a mean of 1.4 ± 2.0 MP sample^{-1}, resulting in an LOQ which was an order of magnitude higher than the mean microplastic concentration (21.6 MP sample^{-1}). The LOD/LOQ will be reduced when samples are analysed in facilities with low background contamination of microplastics (Namieśnik, 2002). Similar to other environmental contaminants (e.g., metals, PFAS), this is especially important in samples where it would be expected to isolate low concentrations of MP from the sample matrix, for example predatory fish (Walkinshaw et al., 2020). Results of this study thereby reiterate that such samples should be analysed in an ultra-trace clean laboratory, theoretically reducing the LOD, and allowing for trace quantities of microplastics to be detected (Belontz and Corcoran, 2021).

When applying the Welch t-test to the environmental datasets, only the GBR sediments data conformed to the assumption of normality. Therefore, the barramundi, scallop muscle and scallop GIT datasets were all analysed with Mann-Whitney tests (Table S13). Results of the statistical analysis mirrored the results the LOD/LOQ method adjustments, where the abundance of microplastics isolated from barramundi ($p < 0.05$) and GBR sediments ($p < 0.0001$) were statistically distinct from the background contamination found in the controls (Fig. 7E, Fig. 7F). Whereas in both the scallop GIT and scallop muscle datasets, the abundance of microplastics in the samples was indistinguishable from the abundance of microplastics in the controls ($p > 0.05$, (Fig. 7G, Fig. 7H).

3.6. Further control analysis considerations

There are still many studies that neglect to use control data to correct sample data, despite reporting the use of controls throughout the analytical process, this is particularly concerning as many of these studies do in fact isolate microplastic from the controls (e.g. Corradini et al., 2019). This is often coupled with the use of an insufficient number of controls, which, based on the result of this study, are unlikely to capture the true heterogeneity of possible contaminants, and thus underestimate the number of contaminating particles within the corresponding samples. For example, (Corradini et al., 2019) used one procedural control per 20 samples, with a total of 100 samples analysed. Lastly, insufficient controls which do not actually encapsulate contaminant sources during laboratory analysis i.e., open Petri dishes (Hermsen et al., 2018; Zhang et al., 2021), are still often used in microplastics research. These practises increase the likelihood of over reporting microplastic pollution in the environment.

Most methods examined in this study were not robust enough to account for the heterogeneity present within microplastics datasets. Despite two of the six final non-statistical data adjustment methods including the Size variable, an argument can be made that as an

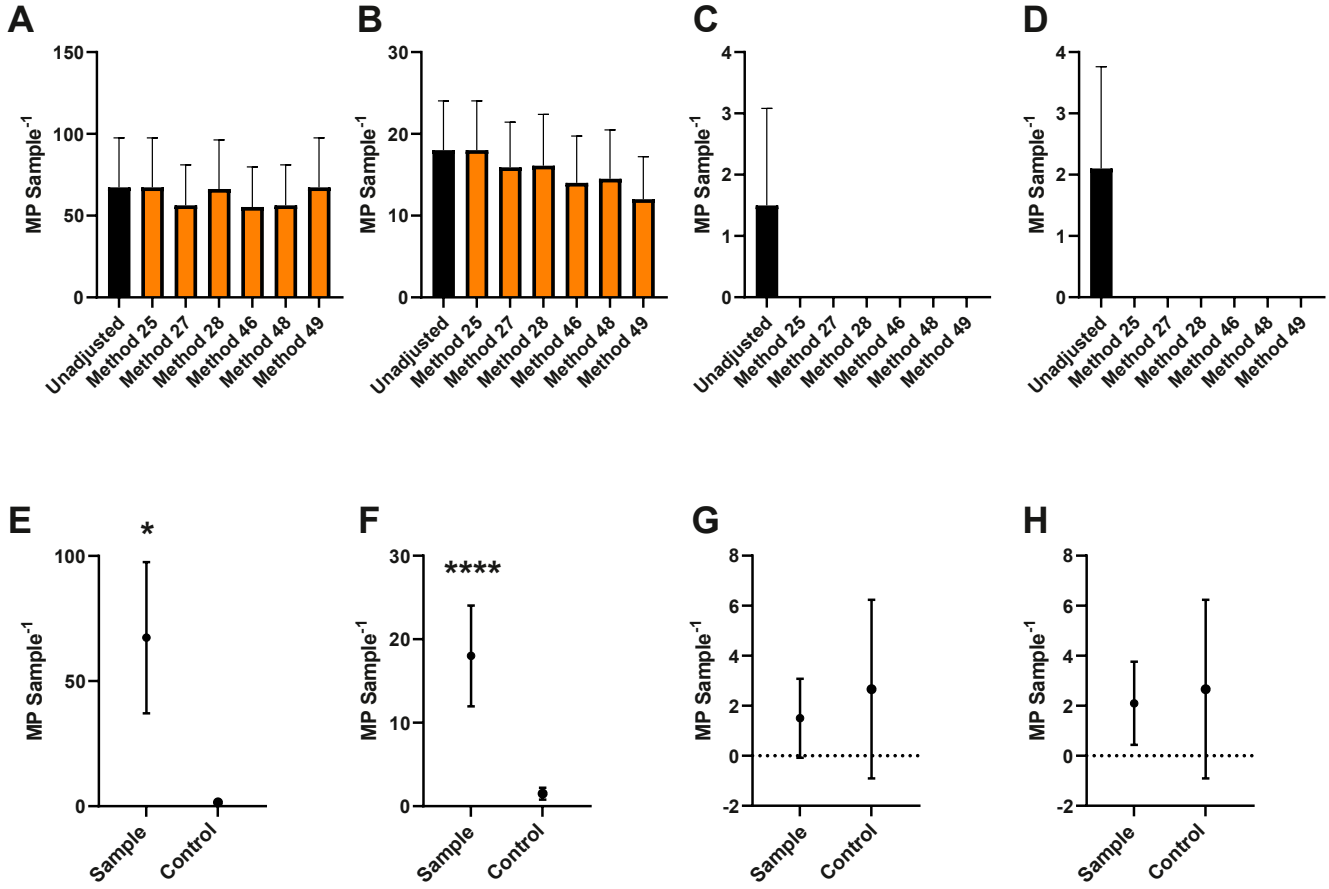

Fig. 7. Environmental datasets with methods 25, 27, 28, 46, 48 and 49 applied A) barramundi muscle B) GBR sediments C) scallop GIT and D) scallop muscle; and analysed statistically using t-tests (F) or Mann-Whitney tests (E, G,H). E) barramundi muscle F) GBR sediments G) scallop GIT and H) scallop muscle. *Symbols denote statistically significant differences between samples and controls with *($p < 0.05$) and * ** * ($p < 0.0001$).

explanatory variable, size is arbitrary and is not a distinguishing feature specific to a contamination source. For example, PBTP isolated from the dummy samples were all derived from the same source (Schott bottle lids) yet the fragment size ranged from 109.6 μm to 1013 μm. Furthermore, there is every reason to assume items will break or warp during the sample processing steps (Jaafar et al., 2020; Pfohl et al., 2021; Stock et al., 2019), especially when recovering weathered plastics from environmental samples (Lenz et al., 2021; Masry et al., 2021). This variation was also observed for the cellulose particles in this study. Some were clearly of fibrous origin, however, became either entangled or fused to form an irregular fragment shape, and thus were assigned as fragments (Fig. S14). Finally, when assigning shape of putative microplastics, often the small size of particles can impede an accurate characterisation of the shape, with shape classification often subjective to the analyst (Kooi and Koelmans, 2019). One option to overcome these changes in size or shape during processing would be to use a correction method that does not utilise categorical variables, such as Method 25, (LOD/LOQ), Method 46 (combination spectral similarity with LOD/LOQ) or Method 50 (*t*-test).

The authors are unaware of other methods currently used in microplastics studies to correct samples data. However, the dummy dataset created for this study can be used to test method that may arise in the future. The methods tested here are but a representative of the total array of possible methods that could be employed to correct microplastics data. Out-of-the-box thinking may be required to meet this challenge, and methods used to analyse other environmental contaminants could be considered. For example, asbestos fibres are particulate contaminants, and like microplastics, environmental monitoring of asbestos is often carried out using filters (Gaggero et al., 2017; NOHSC, 2005; WHO, 1997). Analysis includes a processing step to establish a maximum allowable particle count within the controls. When this threshold is exceeded, the entire sampling and analytical procedure is examined to locate the causes of contamination. Where the maximum allowable count is exceeded, and the control count exceeds 10 % of the sample count, the entire batch of samples must be disregarded. These asbestos QA/QC methods may also be applicable to microplastics. Adopting control and control methods from established monitoring fields could be considered for microplastics monitoring until analyte specific methods are developed.

4. Conclusions

Establishing an accurate and reliable method to adjust microplastic data for extraneous contamination has proven challenging, particularly for samples with low concentrations of microplastics. However, over the past decade, microplastic research methods have significantly evolved and studies which fail to include blanks and controls alongside environmental monitoring should be treated with caution. They run the risk of erroneously reporting laboratory contamination as environmental contamination and therefore may be considered less reliable than those which do adhere to strict environmental monitoring protocols. Moving forward, careful consideration should be given when interpreting these studies alongside more reliable ones. Overall based on the results of this study, the following recommendations are provided.

1. It is recommended to use LOD/LOQ methods or statistical analysis methods (means comparisons e.g., t-test) to analyse microplastic control data, or if other methods are chosen, adequate justification should be given. The final seven methods presented here lay the foundation for further method harmonisation.
2. As demonstrated here, microplastic data is extremely variable even amongst almost identical samples. The adjustment method needs to be flexible enough to account for this variability without compromising on data resolution. One obvious way to assist with this process is to use clean facilities. But as demonstrated previously (Belontz and Corcoran, 2021), this is not always enough to remove contamination.
3. Regardless of the correction method applied, there needs to be an adequate number of controls to be able to account for the variation in microplastic contamination. Although this is dependent on the laboratory environment and sample matrix, at the very minimum each batch of samples extracted should have corresponding controls. Exploration of the background contamination using dummy samples, as done here, may be a useful tool to determine the number of controls required for a specific laboratory environment.
4. As demonstrated in this study, the correction method can drastically change the dataset interpretation, thus adequate information is needed to describe the method chosen. Ideally enough information should be given to facilitate replication, and equations should be stated.

Environmental implication

Microplastics have proliferated in every environmental compartment and are proposed to be a planetary boundary threat. Microplastics are a diverse suite of contaminants, encompassing a heterogenous array of physical characteristics, while also potentially containing a suite of chemical additives, and sorbed contaminants. This complexity makes quantification challenging.

The study provides guidance for data analysis in environmental studies quantifying microplastics within various biological and abiotic matrices. Data analysis within current environmental studies are often opaque, particularly the analysis of controls and blanks. The work facilitates robust analysis and consistency across different studies, contributing to the goal of microplastic method harmonisation.

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CRediT authorship contribution statement

AD, CM and MS conceptualised the study. AD collected the data and analysed samples. AD and MS analysed the data. All authors contributed to interpretation of the analysis. AD wrote the manuscript draft. All authors contributed to writing the manuscript. AD and MS both contributed equally to this study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data and code is available at https://apps.aims.gov.au/metadata/view/a7e5a691-5b29-4ba1-b014-b06bbf10250b.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2022.130218.

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